REMARKS

Claims 1-7 are all the claims pending in the application; each of the claims has been rejected.

Paragraph 0028 of the specification has been amended to insert sequence identifiers for polynucleotides previously only referred to by GenBank accession numbers. The nucleotide sequence encoding the *S.* Typhi ClyA protein has been included in the revised Sequence Listing (SEQ ID NO:21) being filed herewith. The nucleotide sequence is identical to that shown at nucleotides 516-1430 of SEQ ID NO:1.

Paragraph 0029 of the specification has been amended to correct an error in a reference to subject matter known in the art. Wallace et al. (*Cell* 100:265-276 (2000)) teach that amino acid substitutions of V185S, A187S, and I193S of the *E. coli* HlyE protein resulted in a loss of hemolytic activity. Similarly, Atkins et al. (*J. Biol. Chem.* 275:41150-41155 (2000)) teach that amino acid substitution of G180V of the *E. coli* HlyE protein resulted in a loss of hemolytic activity. Copies of both documents are included with the IDS filed herewith. As paragraph 0029 erroneously referred to ClyA in place of HlyE, it has been corrected.

Paragraph 0030 of the specification has been amended to correct an obvious misspelling.

Paragraph 0044 of the specification has been amended to correct a sequence identifier.

Paragraph 0100 of the specification has been amended to insert a sequence identifier for a sequence included in the original Sequence Listing.

Claim 1 has been amended to recite specific export proteins. Support for the amendment is found in claim 4 originally filed with the application, and in paragraph 0028 of the specification.

Claims 1, 2 and 3 have been amended to recite gram-negative bacteria. Support for this amendment is in the provisional application in that only gram-negative bacteria are taught for use in the claimed method.

The remainder of the amendments to the claims are being made to place the claims more fully in U.S. claim format.

New claims 21 and 23 are supported by the specification, such as in paragraph 0076 where collection of proteins from culture media is discussed.

New claim 22 is supported by the specification, such as in the example encompassing paragraphs 0099-0102.

New claim 24 is the same as cancelled claim 6, but placed in independent format.

New claims 25 and 26 parallel claims 1 and 7. Support for the specific export proteins recited in claim 25 may be found in claim 4 originally filed with the application, and in paragraph 0028 of the specification.

A revised Sequence Listing is enclosed herewith, including sequences referred to in the specification (paragraph 0028) by GenBank accession numbers.

No new matter has been added. Entry of the amendment and the revised Sequence Listing is respectfully requested.

I. Claim to Priority

At page 2 of the Office Action, the Examiner states that Applicant's claim to domestic priority under 35 U.S.C. §119(e) is acknowledged, but that the provisional application under which priority is claimed fails to provide adequate support for claims 1-7.

Applicants note that claim 1 is drawn to a method for expressing a polynucleotide in bacterial cells using an expression vector encoding an export protein fused to a protein of interest. Such a system is clearly disclosed in the priority application (60/252,516). For example, pages 8-9 of the provisional application detail the construction and testing of an expression vector comprising a *clyA-bla* fusion. This example provides support for each element of claim 1.

Support for the use of *S.* Typhi and *E. coli*, as recited in claims 2 and 3, respectively, may be found in the paragraph bridging pages 9 and 10 of the provisional application.

Use of the *S.* Typhi ClyA protein, as recited in new claim 22, may again be found at pages 8-9 of the provisional application. The discussion of homologous export proteins from other bacterial supports the use of the other ClyA proteins recited in claim 1.

The last paragraph on page 5 of the provisional application provides support for the amino acid sequence of the *S.* Typhi ClyA protein. The size of the protein is provided (34.1 kDa), the size of the open reading frame encoding the protein is provided (918 bp) and the sequence of primers that may be used in isolating the cDNA encoding the ClyA protein are provided (pages 6-7 of the provisional application). Given this information, one of ordinary skill in the art would be able to isolate the cDNA encoding the *S.* Typhi ClyA protein recited in claim 5.

Use of the method to express an antigen, as recited in claim 7, is discussed at page 6, lines 14-18, of the provisional application.

II. Information Disclosure Statement

At page 2 of the Office Action, the Examiner states that the non-patent documents cited on the document list submitted with the IDS on July 25, 2002, were not provided.

Applicants apologize for the oversight and enclose herewith each of the non-patent documents cited in the IDS filed on July 25, 2002, along with a revised IDS (and fee) and reference list. Applicants respectfully request return of an initialed and signed copy of the reference list, indicating consideration of each of the listed documents by the Examiner.

III. Specification

At page 3 of the Office Action, the Examiner states that the specification includes an improper attempt to incorporate by reference GenBank sequences.

Enclosed herewith is a revised Sequence Listing containing each of the sequences referenced in paragraph 0028 (pages 7-8) of the specification. The instant amendment also amends the specification to insert sequence identifiers into the specification, and corrects errors in references to specific sequences in the sequencing listing.

In view of the revised Sequence Listing, and the amendments to the specification, the application now properly meets the requirements of 37 C.F.R. §§1.821-1.825.

IV. Claim Rejection Under 35 U.S.C. §112

A. At page 3 of the Office Action, claims 1-7 are rejected under 35 U.S.C. §112, first paragraph, as being non-enabled.

The Examiner states that the specification is only enabling for a method of producing a fusion protein, comprising the fusion of the *S.* Typhi ClyA protein and a protein of interest, in *S.*

Typhi. The Examiner does not find the use of other *clyA* genes or the use of other bacteria to be enabled.

Applicants include herewith an amendment to claim 1, and new claim 25, to define the specific export proteins that may be used in the methods of claims 1 and 25 (the export protein is the *S.* Typhi ClyA protein, the *E. coli* HlyE protein, or the *S. paratyphi* ClyA protein. Also included herewith is an amendment to the claims to define the bacteria that may be used in the claimed method (gram-negative bacteria).

The skilled artisan would understand based on the disclosure of the instant application, that the *E. coli* HlyE protein and the *S. paratyphi* ClyA protein could be used in place of the *S.* Typhi ClyA protein. As explained in paragraphs 0022-0030 of the specification, specifically paragraph 0028, each of the proteins are members of the HlyE family. HlyE family members are export proteins that are exported out of bacteria in which they are expressed. The skilled artisan would understand that each of the HlyE family members may be used in the method of the present invention.

Furthermore, as discussed in the specification, such as in paragraph 0023, ClyA proteins are exported from bacteria other than *S.* Typhi, such as *E. coli*. The skilled artisan would understand that other gram-negative bacteria can be used, such as *Shigella*, in place of *S.* Typhi and *E. coli*, in the export of ClyA fusion proteins.

As to new claim 24 (corresponding to canceled claim 6), Applicants include herewith a Declaration Under 37 C.F.R. §1.132 by Dr. James Galen, the inventor of the method recited in the pending claims. As discussed therein, the *E. coli* HlyE protein, having three of the mutations recited in claim 24, is exported from bacterial cells expressing the mutated polynucleotide. In

view of the high degree of homology between the four export proteins recited in the claims (see Appendix I), the skilled artisan would expect that mutation of the amino acids at the same positions in *S.* Typhi ClyA would also produce an attenuated protein that maintains its export abilities.

In view of the amendments made herein, and the expert testimony provided herewith, Applicants respectfully assert that the claims are fully enabled for their entire scope. Accordingly, Applicants respectfully request reconsideration and withdrawal of this rejection.

B. At page 7 of the Office Action, claims 1-7 are rejected under 35 U.S.C. §112, second paragraph, as being indefinite.

As to claim 1, the Examiner states that the term "the culture medium" lacks antecedent basis, and that claim 1 is technically incorrect as it recites a polynucleotide fused to a polypeptide.

As to claim 2, the Examiner states that the bacterial cell employed uses terminology inconsistent with that used in the art.

As to claim 4, the Examiner states that the first use of an abbreviation must be spelled out.

Applicants include herewith amendments to the claims to address each of the issues raised by the Examiner. With regard to the used of *S.* Typhi, Applicants respectfully note that "*S.* Typhi" is the correct abbreviation for *Salmonella enterica* serovar Typhi.

V. Claim Rejection Under 35 U.S.C. §102

A. At page 9 of the Office Action, claims 1, 3, 4 and 7 are rejected under 35 U.S.C. §102(b) as being anticipated by Ikonomidis et al. (J. Exp. Med. 180:2209-2218, 1994).

The Examiner states that Ikonomidis et al. teaches a fusion between listeriolysin O (LLO) and nucleoprotein (NP) that is expressed and secreted by *Listeria monocytogenes*, and that such teaching falls within the scope of the rejected claims.

As discussed above, the claims have been amended such that the export protein portion of the fusion protein is one of the following: the *S.* Typhi cytolysin A (ClyA) protein, the *E. coli* (HlyE) protein and the *S. paratyphi* ClyA protein. In addition, the bacteria used in the claimed method are gram-negative bacteria.

In contrast, Ikonomidis et al. teaches the use of *Listeria monocytogenes* listeriolysin O as the export protein, and the use of *Listeria monocytogenes* as the bacteria which expresses the fusion protein.

As the teachings of Ikonomidis et al. are limited to the use of an export protein from *Listeria monocytogenes* and the use of a gram-positive bacteria (see first sentence, column 1, page 2209), Ikonomidis et al. does not teach each element of the rejected claims. Accordingly, Ikonomidis et al. does not anticipate any of the rejected claims and Applicants respectfully request reconsideration and withdrawal of this rejection.

B. At page 9 of the Office Action, claims 1, 3, 4 and 7 are rejected under 35 U.S.C. §102(b) as being anticipated by Gentschev et al. (Behring Inst Mitt. 98:103-113, 1997).

The Examiner states that Gentschev et al. teaches a fusion between *E. coli* hemolysin hlyA and a protein of interest that is expressed and secreted by *E. coli*, *Salmonella typhimurium*, and *S. dublin*, and that such teaching falls within the scope of the rejected claims.

As discussed above, the claims have been amended such that the export protein portion of the fusion protein is one of the following: the *S.* Typhi cytolysin A (ClyA) protein, the *E. coli* (HlyE) protein and the *S. paratyphi* ClyA protein.

In contrast, Gentschev et al. teaches the use of the hemolysin A protein of *E. coli* as the export protein. As discussed in the second paragraph of the introduction of Wallace et al. (*Cell* 100:265-276 (2000) enclosed herewith, hemolysin A and hemolysin E of *E. coli* are unrelated proteins.

As the teachings of Gentschev et al. are limited to the use of the hemolysin A protein of *E. coli*, and do not teach the use of the hemolysin E protein of *E. coli* (as recited in the pending claims), Gentschev et al. does not teach each element of the rejected claims. Accordingly, Gentschev et al. does not anticipate any of the rejected claims and Applicants respectfully request reconsideration and withdrawal of this rejection.

VI. Claim Rejection Under 35 U.S.C. §103

At page 10 of the Office Action, claims 1-4 and 7 are rejected under 35 U.S.C. §103(a) as being unpatentable over Gentschev et al. in view of Curtis et al. (US Patent No. 5,387,744, issued February 7, 1995).

The Examiner states that while Gentschev et al. does not teach expression of the fusion protein in *Salmonella typhi* (claim 2), it would have been obvious to use *Salmonella typhi* based on the teachings of Curtis et al., in the method of Gentschev et al., to arrive at the instant invention.

As discussed above, the teachings of Gentschev et al. are limited to the use of the hemolysin E protein of *E. coli*, and do not teach the use of the hemolysin A protein of *E. coli* as

recited in the amended claims. Therefore, Gentschev et al. does not teach each element of the rejected claims. Curtis et al. does not teach a method for producing a fusion protein using a bacterial hemolysin export protein linked to a protein of interest as recited in the amended claims.

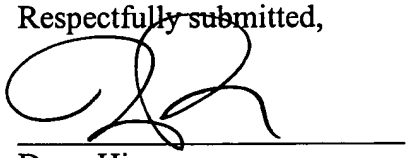
As neither Gentschev et al. or Curtis et al., alone or in combination, teach or make obvious the claims as amended, Applicants respectfully request reconsideration and withdrawal of this rejection

VII. Conclusion

In view of the above, reconsideration and allowance of this application are now believed to be in order, and such actions are hereby solicited. If any points remain in issue which the Examiner feels may be best resolved through a personal or telephone interview, the Examiner is kindly requested to contact the undersigned at the telephone number listed below.

The USPTO is directed and authorized to charge all required fees, except for the Issue Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any overpayments to said Deposit Account.

Respectfully submitted,



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